Microbial host interactions

Foodborne *Salmonella* ecology in the avian gastrointestinal tract

K.D. Dunkley [a,1], T.R. Callaway [b], V.I. Chalova [a,2], J.L. McReynolds [b], M.E. Hume [b], C.S. Dunkley [a,3], L.F. Kubena [b], D.J. Nisbet [b], S.C. Ricke [a,*]

[a] *Department of Poultry Science, Texas A&M University, College Station, TX 77843-2472, USA*
[b] *USDA-ARS, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, TX 77843, USA*

A B S T R A C T

Foodborne *Salmonella* continues to be a major cause of salmonellosis with *Salmonella* Enteritidis and *S.* Typhimurium considered to be responsible for most of the infections. Investigation of outbreaks and sporadic cases has indicated that food vehicles such as poultry and poultry by-products including raw and uncooked eggs are among the most common sources of *Salmonella* infections. The dissemination and infection of the avian intestinal tract remain somewhat unclear. *In vitro* incubation of *Salmonella* with mammalian tissue culture cells has shown that invasion into epithelial cells is complex and involves several genetic loci and host factors. Several genes are required for the intestinal phase of *Salmonella* invasion and are located on *Salmonella* pathogenicity island 1 (SPI 1). *Salmonella* pathogenesis in the gastrointestinal (GI) tract and the effects of environmental stimuli on gene expression influence bacterial colonization and invasion. Furthermore, significant parameters of *Salmonella* including growth physiology, nutrient availability, pH, and energy status are considered contributing factors in the GI tract ecology. Approaches for limiting *Salmonella* colonization have been primarily based on the microbial ecology of the intestinal tract. *In vitro* studies have shown that the toxic effects of short chain fatty acids (SCFA) to some *Enterobacteriaceae*, including *Salmonella*, have resulted in a reduction in population. In addition, it has been established that native intestinal microorganisms such as *Lactobacilli* provide protective mechanisms against *Salmonella* in the ceca. A clear understanding of the key factors involved in *Salmonella* colonization in the avian GI tract has the potential to lead to better approach for more effective control of this foodborne pathogen.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

1.1. Salmonella nomenclature

Salmonella are gram-negative bacteria consisting of non-spore forming bacilli and are a member of the family *Enterobacteriaceae*. The nomenclature of *Salmonella* is quite complex and is based on both serotype and subspecies names. For example, *Salmonella enterica* subspecies *enterica* serotype Enteritidis is shortened to *Salmonella* serotype Enteritidis or *Salmonella* Enteritidis [1]. *Salmonella* can be further subdivided onto biotype and phase type with biotype being a biochemical variation between two microorganisms of the same serotype, whereas the phase type is based on the differences in susceptibilities of two microorganisms of the same serotypes to a lytic bacteriophage [2,3]. *Salmonella* are also classified by three distinct types of antigens including somatic O, flagella H, and capsular Vi antigens. Antigens have been used to isolate and identify more than 2500 serotypes of *Salmonella* [4]. There are two species of *Salmonella*, namely *S. bongori* and *S. enterica*. *S. enterica* is divided into six subspecies including *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. The most common O-antigen serogroup within *S. enterica* subspecies are A, B, C1, C2, D, and E strains. This serogroup is numerically the most significant and causes approximately 99% of *Salmonella* infections in humans and warm-blooded animals [5].

* Corresponding author. Current address: Departments of Poultry Science and Food Science, and Center for Food Safety, IFSE, University of Arkansas, 2650 N. Young Ave., Fayetteville, AR 72704-5690, USA. Tel.: +1 479 575 4678; fax: +1 479 575 6936.
  E-mail address: sricke@uark.edu (S.C. Ricke).
[1] Current address: Department of Natural Sciences, Albany State University, Albany, GA 31705, USA.
[2] Current address: Departments of Poultry Science and Food Science, and Center for Food Safety, IFSE, University of Arkansas, Fayetteville, AR 72704-5690, USA.
[3] Current address: Department of Poultry Science, The University of Georgia, Tifton, GA 31793, USA.

1.2. Epidemiology

Foodborne salmonellosis is responsible for over 600 deaths and 1.4 million illnesses in the US annually [6] and the costs for medical care and loss of productivity can range anywhere from $464 million to $2.3 billion [7]. In 1999, 22% of all culture-confirmed *Salmonella* infected individuals were hospitalized [8]. *Salmonella* have also been commonly associated with foods such as raw meat, poultry, eggs, and dairy products and cause a large fraction of the food-related deaths in the US annually [9]. In Europe, the number of human cases was reported to be greater than 100,000 in 1997 [10]. In the past few years, the incidence of salmonellosis has shown a significant decrease across Europe (73,000 cases in 2001) and in the US since 1996 [10,11]. Approximately 60% of human cases reported to the CDC (in 2001) were caused by four serotypes including *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg [11]. *Salmonella* cases associated with certain serotypes, however, increased in 1999 and were accompanied by decreases in *Campylobacter jejuni*, *Shigella*, and *Escherichia coli* O157:H7 [8]. In 2004, new incidences of *S.* Newport and *S.* Javiana increased up to 41% and 167%, respectively, over the 1996–1998 baseline period [12].

2. *Salmonella* in poultry

A variety of investigations of outbreaks and sporadic cases have indicated that food vehicles identified as the most common source of *Salmonella* infections are poultry and poultry by-products, including raw and uncooked eggs [13–19]. *Salmonella* cause asymptomatic intestinal infections in birds but acute outbreaks exhibiting clinical disease along with high levels of mortality occur in chicks younger than 2 weeks old [20–22]. Egg shells can be contaminated with *Salmonella* as a result of intestinal passage and the ability to penetrate into the avian egg [23]. Pullorum disease, for example, is caused by *S.* Pullorum and is spread from an infected parent bird via the egg to the chicken. While clinical signs are variable and non-specific, the outcome is an excessive number of dead-in-shell chicks and deaths shortly after hatching. *Salmonella* can be highly invasive in laying hens leading to systemic infections that can potentially be deposited in the internal contents of eggs by transovarian transmission following colonization of the intestinal tract [24–30]. *S.* Enteritidis, in particular, has shown a greater ability to colonize the vaginal epithelium of laying hens compared to other serotypes [31]. Birds that are asymptomatic carriers may facilitate the spread of disease infections among flock [32,33]. *S.* Enteritidis contaminated eggs have proven to be extraordinarily difficult to detect internally and unless bacterial populations exceed $\log_{10}$ 9.0 per egg, no distinct changes in appearance or odor are usually observed [34]. *S.* Gallinarum is excreted in the feces of infected birds and can persist in feces for at least 1 month and longer in carcasses.

Even though *Salmonella* pathogenesis has been well characterized in the mammalian model, there is limited information on specific mechanisms in the avian species. Light and electron microscopic examinations of intestines taken from chickens experimentally infected with various *Salmonella* species demonstrated similar cellular responses to these organisms, including the influx of heterophils and macrophages to the luminal surface of the intestine [35,36]. Heterophils are considered to be the avian counterpart to mammalian neutrophils in their action as tissue phagocytes, and their importance to host defense against bacterial infections is well known [37]. The capabilities of the heterophils and avian macrophages to kill *Salmonella* have been demonstrated through bactericidal assays performed *in vitro* [38]. If *Salmonella* are not cleared by the immune system, colonization of the intestine occurs, and they are able to move through and colonize other cells by inducing them to take up the bacteria [39]. Studies have shown that at least *Salmonella* used to experimentally infect birds will migrate from the intestine to the liver, spleen, and ovaries [25,27,29,30,36,40,41]. This indicates that the pathogenesis of *Salmonella* in experimental avian model infections involves a sequential dissemination in the internal organs that is similar to what has been established in the mammalian model.

3. Microbial ecology in the avian GI tract

3.1. Avian GI tract and indigenous microflora

The lower gastrointestinal (GI) tract of most animal species including poultry is normally populated by large numbers of microorganisms [42], and through various competitive niches and virulence capabilities, some are able to survive. The capabilities of microorganisms associated with the mucosa of the GI tract to withstand the flow rates of food material is essential for the development of protective mechanisms such as surface mucus colonization [43], deep mucus, development of specialized insertional structures [44], and crypt association [45] by specific adhesions [46]. Changes in the passage rates that are representative of dilution rates can alter the limiting nutrients and therefore could ultimately affect microflora composition in the GI tract ecology.

Historically, the microbial composition of the GI tract of avian species has not been extensively defined compared to what is known about microorganisms in ruminants [47]. There is the perception that the role of microorganisms in chickens is not as important as is the case for ruminants [48]. However, extensive strict anaerobic metabolism including methanogenesis fermentation occurs in birds fed a variety of diets [49–52]. The ceca are the major fermentation sites in the GI tract of chickens and contain the largest number of bacteria [42,53–55]. Over 200 different bacteria have been isolated and characterized [56], and these bacteria are known to be influenced by various factors including diet, health, and age. However, Zhu et al. [57] indicated that only 10–60% of microorganisms in the ceca could be propagated using anaerobic culture techniques.

3.2. Methods for studying avian GI microbial ecology

Continuous culture (CC) techniques historically have provided *in vitro* models to study GI tract metabolism and fermentation of microorganisms [58–64]. A typical CC experiment involves a chemostat apparatus that simulates specific GI tract physical and chemical properties and consists of an afferent inlet which inputs substrates and buffer and an efferent outlet that facilitates outflow port of a homogenous mixture of microorganisms, fermentative metabolites and substrates [65]. The growth of bacteria in CC can provide a more accurate reflection and simulate conditions that are closely related to the natural GI ecosystem. Steady state can be attained and significant parameters can be quantified including rate of growth, manipulation of nutrient source, pH status and maintenance energy [58]. This however, depends on how constant the nutrient flows are in the particular GI tract systems. The flow rate (passage rate) may vary for different gut systems including cattle (approximately 80 h) [66], horse (48 h) [67], and chickens (2.5 h) [68]. Furthermore, flow rates depend on the feed composition and texture [69].

Experimentally, CC techniques have been used to simulate the GI tract microenvironment of humans [61,62] and various animal species including chickens [63,64], pigs [70], and ruminants [58]. Studies which model the human colonic ecology [61] demonstrated antagonism of indigenous microflora against enteropathogens from

crude human fecal cultures in anaerobic culture systems. In these studies, it was observed that five human fecal microorganisms provided levels of antagonism that mimic the crude fecal flora in GI tract in the presence of carbon sources such as lactose, sucrose, and starch that were fermentable only by antagonistic bacteria. The enteropathogen (S. Typhimurium) was suppressed to a 10^4 CFU/mL by 5–7 days post-challenge [61]. Parameters such as competition for growth-limiting amino acids and microfloral density are known to contribute to the superior competitiveness of the normal microflora to outcompete and eliminate pathogens [62,71,72]. Using CC techniques, even small variations in media pH (6.17–7.35) were shown to influence S. Typhimurium physiology by altering the bacterial cell parameters [73]. While ammonia release into media was favored by low pH, the increases in pH resulted in higher cell protein concentrations, glucose disappearance, and bacterial ATP yields.

4. *Salmonella* physiology in the avian GI tract

4.1. Salmonella *growth physiology*

Under nutrient limiting conditions, bacteria reach stationary phase of growth rapidly [74]. When this occurs, bacterial replication ceases and cell density begins to decrease. Historically, research has shown that transition into a survival mode during stationary phase is a more physiologically controlled event by bacteria than previously thought [74,75]. Cessation of growth can be caused by many environmental factors, including acid pH, osmotic stress, heat shock, and redox potential [76–80].

Considerable attention has been given to nutrient starvation with a primary focus on carbon, nitrogen, or phosphorous sources because these are identifiable with already highly characterized genetic changes that occur in bacteria including S. Typhimurium [81]. Ševčík et al. [82] demonstrated that under anaerobic conditions when electron acceptors are scarce, the stationary phase of S. Typhimurium growth may be reached not only by nutrient deprivation but due to a limited availability of electron acceptors such as oxygen. When Salmonella became exposed to such conditions upon infection of a susceptible host, they multiplied rapidly and reached a density of 10^8 CFU/g cecal content [82].

The utility of animal cell culture has become a popular model for studying adhesion and penetration through epithelial cells by Salmonella [83,84]. It has been shown that an adhesion-invasion-deficient mutant of Salmonella is largely controlled by genetics and multiple chromosomal loci [85,86]. Invasion is genetically mediated [87] by several invasion genes found on a 40 kb Salmonella pathogenicity island 1 (SPI 1) located between *flhA* and *mutS* chromosomal genes at centisome 63 on the S. Typhimurium chromosomes [88]. Rodriquez et al. [89] observed that S. Typhimurium incubated in the presence of high osmolarity and low oxygen for 8 h exhibited reduced *hilA* expression during the exponential growth phase. However, at stationary phase (3 h post-inoculation), there was an apparent increase in *hilA* expression most probably due to less optimal growth conditions such as limited nutrients, low oxygen tension, and other stresses created after the first 2 h in the medium. Cell-association and invasion of S. Typhimurium into cultured epithelial cells may also be influenced by short chain fatty acids (SCFA) as a function of both SCFA concentration and pH of the media [90–92].

4.2. Genetics of Salmonella

The central regulator of stationary phase is expressed by *rpoS* [80,93] which is responsible for the induction of a specific subset of bacterial genes expressed during stress. *RpoS* is known to be positively regulated by a starvation specific molecule ppGpp [78] accumulated as a part of the stringent response. In addition, induction of sigma factor can alter the efficiency of metabolism including reduction in cellular concentration of cAMP [94] and UDP-glucose. RpoS is an alternative sigma factor (σ^s) (KatF) which has been demonstrated to be essential for stationary phase stress response in Salmonella and E. coli and is an important gene regulator in S. Typhimurium [81,95,96]. *RpoS* encodes an *RNA* polymerase sigma factor (δ^S or δ^{38}) that is known to regulate at least 60 genes in response to environmental signals including various stress conditions, nutrient limitation, osmotic challenge, acid shock, heat shock, oxidative damage, redox potential, and growth in stationary phase [97–102].

S. Typhimurium is an intracellular pathogen, residing in the macrophages upon infection and can be exposed to a wide range of antimicrobial effectors including the phagocyte NAD(P)H oxidase (Phox). An initial oxidative bactericidal phase associated with the production of superoxide anion and hydrogen peroxide is followed by bacteriostatic phase where nitric oxide is produced [103]. The combination of nutrient limitation and stress conditions in the intracellular environment is probably a stimulus for *rpoS* induction [104]. Starvation also increases the intracellular levels of ppGpp which in turn enhances the level of RpoS [105]. This is evident because ppGpp-deficient strains fail to synthesize RpoS as cells enter into stationary phase in a rich medium and under starvation [105]. The major effects of ppGpp induction are not exerted on *rpoS* mRNA abundance or on protein turnover but instead influence translational efficiency [106].

4.3. Acid tolerance response of Salmonella

Salmonella elicit several strategies to avoid or repair damages that are caused by exposure to acid stress. In Salmonella, RpoS is also integrally involved in the development of several low-pH inducible acid defense systems, collectively referred to as acid tolerance response (ATR), that expand the range of pH tolerance [107–111]. There are two major ATR that have been identified and are based on the particular growth phase in which they become induced. The first type, the log-phase ATR system, operates during the exponential growth phase of cells undergoing a rapid transition to low pH [108]. Over 50 acid shock proteins (ASP) are produced during this response [112]. The second type of ATR system is known as a stationary phase ATR and is induced by exposing cells to low pH during stationary phase [111]. In contrast to the log-phase type system, it is induced by the onset of the stationary phase regardless of the pH of the growth. Bearson et al. [113] reported that both ATR systems in S. Typhimurium are able to protect against the two types of acid stress including organic (weak acids) and inorganic acids (low pH). RpoS and Fur are believed to protect against organic acids, whereas PhoP along with RpoS protect against inorganic acid stress.

Bearson et al. [113] demonstrated that *rpoS* in S. Typhimurium encodes for a shock protein (ASP) and its expression is induced 4-fold by transition from normal to acid conditions (pH less than 4.5). The importance of this induction has been demonstrated for S. Typhimurium to initiate and sustain induction of the ATR [110]. It is a complex adaptive response that induces both an σ^s-independent transient ATR which is maximally induced by 20 min of pH 4.4 acid shock but progressively lost during longer adaptation [114] and a σ^s-dependent sustained ATR which can be seen during a longer period of acid environment of 60–90 min [110].

4.4. Response to short chain fatty acids (SCFA)

SCFA are end-products of microbial fermentation in the GI tract of humans and animals and include acetate, propionate, butyrate,

valerate, isovalerate, and isobutyrate [53,115–117]. Previous studies with four species of birds indicated that metabolizable energy obtained from total SCFA production was equivalent to 5–15% of the daily requirement for maintenance [53,118,119]. However, young chickens do not contain a wide diversity of anaerobic bacteria as a dominant fraction of the microflora [120], and low concentrations of acetate (below 70 μmol g^{-1}), propionate (below 8 μmol g^{-1}), and butyrate (below 24 μmol g^{-1}) are expected during the first week of life [54,120,121]. In the first 15 days after hatching, the concentrations of SCFA in the young chick's ceca vary which may explain their low protective efficiency against pathogen colonization [122]. The production of SCFA reaches concentrations which are considered optimal for pathogen exclusion (acetate at 70 μmol g^{-1}; propionate 8 μmol g^{-1}; and butyrate 24 μmol g^{-1}) and stabilizes after chickens reach 15 days of age [120]. Increases in acetate, propionate, and butyrate in ceca have been assumed to lead to a decrease in the viable population of Enterobacteriaceae in ceca of chickens [123]. However, pre-exposure of Salmonella to high levels of various SCFA (100 mM) at neutral pH may enhance survivability by increasing acid resistance and stimulating virulence response [124–127].

SCFA can inhibit Salmonella growth when present in the dissociated form. Van der Wielen et al. [123] demonstrated in a batch fed competitive exclusion (CE) co-culture that acetate, propionate and lactate inhibited Salmonella growth at pH 5.8, but failed to do so at neutral pH. At pH 5.8, the total undissociated SCFA were significantly higher compared to the dissociated form at neutral pH [128]. At a lower pH (5.8), it is thought that SCFA promote bacteriostatic action by increasing the concentration of undissociated acids. Undissociated acids enhance permeability of the cell membrane [129,130] and cause bacteria to lose energy generating capacity in the form of ATP, thus compromising replication [130]. While a bacteriostatic activity was observed on Enterobacteriaceae, the organic acids did not inhibit beneficial GI tract bacteria such as Lactobacillus [120]. McHan and Shotts [131] observed toxic effects of SCFA to some Entrobacteriaceae and in an in vitro study showed a 50–80% reduction in S. Typhimurium in presence of SCFA. Conversely, Kwon and Ricke [124] noted that organic acids played a role in the survivability of acid sensitive pathogens exposed to reduced pH by induction of ATR which is associated with virulence. Therefore, the use of organic acids may need to be somewhat selective and the exposure of the microbial pathogens especially in the GI tract environment of animals to them must be further evaluated to ensure that organic acids are not a confounding factor in their use as antimicrobial agents.

5. Potential for Salmonella control in avian GI tract

5.1. Probiotics

Probiotics are generally referred to as any live microbial feed supplements that benefit the host animals by largely improving intestinal microbial balance [132,133]. Intestinal microorganisms that are recognized as possessing probiotic properties include but are not limited to Lactobacilli and Bifidobacteria spp. They exhibit identifiable beneficial effects for the respective host via promotion of gut maturation and integrity, antagonism against pathogens (Salmonella) and immune modulation [134–136]. The effects of probiotics in poultry also include maintaining normal intestinal microflora by CE, increasing metabolism, decreasing enzymatic activity and ammonia production, as well as an increase in feed intake and the neutralization of digestive enterotoxins [137,138]. Therefore, the overall goal of probiotics intervention is to promote the general growth of healthy microorganisms that are competitive with or antagonistic to enteropathogens [133].

The application of such probiotics has been referred to as the Nurmi concept of CE. Nurmi and Rantala [122] were the first to utilize CE as a viable pathogen-reduction strategy. They demonstrated that Salmonella colonization in juvenile chickens was reduced by the administration of a preparation of gut bacteria originally isolated from healthy adult chickens. Currently, CE approaches essentially involve pathogen-reduction strategy via introduction of a (non-pathogenic) bacterial culture to the intestinal tract of food animals resulting in reduced colonization or decreased populations of pathogenic bacteria in the GI tract [133,139–141]. Over the past three decades, CE cultures have been extensively studied in several laboratories [64,70,139,142–144] with a primary focus on limiting Salmonella colonization in the GI tract of chickens. There have been several efforts designed to understand and reduce their microbial complexity, improve their resistance and identify successful colonization after introduction of the CE culture [47,70,142,143]. CE cultures in which the bacterial composition is unknown are termed undefined, while those of known bacterial composition are referred to as defined CE cultures [70]. An established and mature GI microbial population theoretically occupies all available environmental niches nutritionally, metabolically and physically, making an animal more resistant to colonization by opportunistic pathogen infections [133].

Van der Wielen et al. [120] reported that in adult chickens the microbial population becomes more complex, stable, and better able to resist enteropathogens than their younger counterparts. In the 1990s, there was considerable progress achieved in developing cultures maintained in CC which were shown to effectively control Salmonella colonization when administered to chickens [139,140,145–147]. The inhibitory mechanism against Salmonella colonization has been associated with a reduction in cecal pH, increase in cecal lactic acid and SCFA, competition for attachment sites, competition for growth-limiting nutrients, production of antimicrobial compounds, immunomodulation, and synergistic and antagonistic interaction [61,71,72,128,142,148,149]. It has been stated that CE should be used as a prophylactic treatment rather than a therapeutic agent [150] and should originate from the intestinal content of the animal of interest. For instance, a CE culture for use in chickens must be derived from healthy chickens, likewise for pigs [70]. Administration of a bacterial community to newly hatched chickens can lead to an early colonization of adherent bacteria on the intestinal mucosal surface forming a mat of microorganism occupying environmental niches [151–153]. In the food animal industry, the use of probiotics and CE can be administered as a synbiotic by combining them with external dietary ingredients that will favor the specific growth and establishment of the probiotic bacteria [154,155]. Roller et al. [156] established that while an inulin-enriched oligofructose dietary supplement increased the production of interleukin-10 in Peyer's patches and secretory immunoglobulin (sIgA) in the cecum of rats, the probiotic mixture (L. rhamnosus and B. lactis) affected the immune functions only modestly. The combined application of both supplements resulted in enhanced production of sIgA in ileum and decreased oxidative activity of blood neutrophils. They concluded that simultaneous administration of probiotics and selected dietary supplements may have different effects than when applied separately.

5.2. Prebiotics

Prebiotics can be defined as non-digestible carbohydrate fractions fed in diets that are beneficial to the host by stimulating the growth of one or more bacteria in the GI tract [157,158]. Prebiotics (dietary fibers) are predominantly a constituent of plant cell walls and also consist of non-starch polysaccharides (NSPs) along with

non-carbohydrate compounds including lignin, protein, fatty acid, and wax [159]. Upon ingestion, dietary fiber may influence the GI tract by altering its microbial activities, rate of passage, metabolites, and digestive efficacy [159,160]. Certain dietary fractions including polysaccharides have been identified for their potential to be utilized as prebiotics [161,162], possibly by reducing pH and increasing VFA concentrations [121,148,163]. Beneficial species of Lactobacilli and Bifidobacteria that are considered to be inhibitory towards pathogens are known to be supported by some of these compounds [157].

Some of the more extensively studied prebiotic sources are fructooligosaccharide (FOS), oligofructose and inulin [154,155]. FOS are naturally occurring oligosaccharides that originate from plants such as onions, wheat, barley, and rye and consist of one to three fructose residues attached to a sucrose molecule. When fed to animals, FOS have been shown to impact bacterial populations by promoting the growth of Lactobacillus [164] and Bifidobacterium spp. [165]. Bailey et al. [166] demonstrated reduced susceptibility of broiler chickens to Salmonella invasion after inclusion of FOS in their diets which was explained by a probable shift in gut microorganisms. The efficiency of FOS in the same study was enhanced by a combination with a protective CE culture which resulted in 3-fold reduction of S. Typhimurium chicken colonization compared to chickens given CE alone. Lactobacillus paracasei administered in combination with FOS resulted in a significant increase of Lactobacillus spp., Bifidobacterium spp., total anaerobes, and total aerobes, as well as a decrease in Clostridium and Enterobacterium observed in piglets [167]. In a series of in vitro studies, Donalson et al. [168,169] demonstrated that a combination of FOS, alfalfa and grain, incubated with cecal inoculum exhibited a significant reduction in Salmonella population, while increasing propionate, butyrate, other SCFA, and lactate. However, in vivo work with laying hens was less conclusive indicating some adaptation of the cecal microflora was required [170]. In at least half of the trials, the S. Enteritidis colonization of ovary and liver of hens fed FOS (0.375% and 0.750%, w/w) containing diets were reduced compared to hens subjected to complete removal of feed. Significant decreases in cecal S. Enteritidis counts were also observed in only half of the trials. However, no substantial differences in Salmonella colonization of hens' organs were observed due to FOS. Although the addition of FOS to cereal or high-fiber diets did not improve the production of the total cecal VFA, hens fed high fiber with or without FOS yielded greater cecal lactic acid concentrations than hens subjected to complete removal of feed [170].

5.3. Dietary strategies to limit Salmonella in the avian GI tract

Adding specialized prebiotics may be economically limiting depending on the cost of the original sources of the compounds used, so recent research has focused on examining dietary regimens that elicit similar properties. This has been studied in some detail for certain egg-laying hen management practices in the poultry industry. In particular, molting diets for layer hens have been a focal point for development of these types of diets. Natural molt of hens is associated with the temporary interruption of egg production. Instead, hens utilize their energy in staying warm and growing new feathers [171]. Historically, the shortening of the natural molt and rejuvenation of hen flocks in poultry industry were achieved by withdrawal of feeds [172]. Feed deprivation was a procedure employed to achieve a rapid and economical new egg-laying cycle [173,174] and could last anywhere from 4 to 14 days [172,175]. However, this method, although possessing several management advantages, has become less popular due to a variety of animal and food safety issues [28,29,176–178]. It was suggested that the avian microbial ecology is altered during dietary stresses

such as feed removal which in turn can lead to higher vulnerability of the host to pathogen infection and colonization [28,40,127,179]. Changes in dietary composition of the GI tract of poultry during feed withdrawal clearly have negative consequences on microbial population.

It has been proposed that dietary fiber can be utilized preferentially by Lactobacillus and Bifidobacteria species which leads to the production of lactic acid and SCFA, resulting in the maintenance of normal microbial populations, low pH and also prevents the establishment of Salmonella in the GI tract [180–182]. Studies have shown that feed deprivation can alter the hen's immune system and physiological status [183–189]. Laying hens also become more susceptible to pathogen infection including Salmonella spp. with molted hens shedding significantly more S. Enteritidis in their feces [26,27,190], and higher levels of S. Enteritidis invasion in their internal organs including liver, spleen, and ovaries [25,27,40]. These findings suggested that complete removal of feed promotes pathogen invasion in molted hens.

Diets that regulate the passage rate by slowing it down could be advantageous since this mechanism may prolong fermentation which in turn increases metabolites needed to maintain GI tract integrity. The altering of passage rate (flow rates) represents changing the amount of digesta that passes a point along the GI tract in a given time [191]. Passage rate may vary in different segments of the GI tract and is dependent on the feed composition and texture [192,193]. Adequate feed retention time is essential especially in the ceca in order to encourage microbial degradation for longer periods of time [194] leading to the production of important metabolites, which subsequently maintain the integrity and an optimal range of microbial diversity.

Several high-fiber dietary approaches have been utilized as alternative molting diets to expedite an additional laying cycle for hens. This includes insoluble plant fiber such as grape pomace [195], cotton meal [196], wheat middling [197], and alfalfa [30,41,179,185,186,198–203]. Studies by Tsukahara and Ushida [204] demonstrated that feeding a plant protein-based diet to chicks generated a higher concentration of SCFA than a diet based on animal proteins and implied that the difference in SCFA concentration was due to a higher concentration of the dietary fiber component in the plant diet. In addition, it has been reported that certain microorganisms that are indigenous to the GI tract of poultry have the potential to hydrolyze dietary fiber into oligosaccharides and other low molecular weight carbohydrates which leads to production of SCFA [48,49,127,205,206].

Alfalfa is one of the more extensively studied high-fiber dietary sources in poultry. It has been widely used as animal feed and as a high-fiber feed source [207–209]. It is relatively high in protein exhibiting one of the slowest rates of passage (more than 24 h) through the avian system and components such as saponins can influence digestion and consumption of feed [210,211]. Alfalfa is well balanced in amino acids and rich in vitamins, and contributes to the desirable yellow color to carcasses and egg yolks when fed to chickens as a dietary supplement [208,212]. In addition, alfalfa may have advantages associated with the fermentation properties by cecal microflora that are capable of limiting in vitro growth of S. Typhimurium and has been shown to limit in vivo S. Enteritidis colonization in laying hens [30,41,170,202,203]. An in vitro study examined the fermentation of alfalfa and layer feed incubated with chicken cecal content in rumen fluid using nitrocompounds and indicated that both feed materials influenced SCFA production with acetate being the predominant component [50]. It was observed that incubation with the methane inhibitors nitroethanol and 2-nitropropanol produced significantly higher propionate than nitroethane, while layer feed produced more butyrate than alfalfa. The addition of nitropropanol to layer feed incubated with cecal

contents was suggested to promote gram-positive, saccharolytic SCFA-producing bacteria especially *Clostridium* spp. which is a predominant group in the ceca of chickens [57,213,214]. High-fiber feed substrates (soybean meal, soybean hull, beet pulp, wheat middlings, ground sorghum, cottonseed meal, alfalfa, and different ratios of alfalfa and commercial layer ration) have also been observed to influence microbial diversity and stimulate SCFA production when incubated with chicken cecal inocula *in vitro* [206]. While isobutyrate and isovalerate were barely detectable, acetate production was pronounced, followed by propionate and butyrate.

In order to derive maximum benefit from fermentable high-fiber prebiotic sources, physical modification may also be necessary to derive uniform particle size. Coarsely ground mash over whole grain wheat has been demonstrated to be effective on the physiological function on GI tract of broiler birds. It has been shown that an increase in feed structure caused an increase in gizzard size [215–218]. A reduction in gizzard pH and an increase in small intestinal pH were observed with an increase of the grain particle size [217,218]. The particle size of feed structure is also known to influence *Salmonella* numbers [193]. It has been demonstrated that pigs fed a coarse non-pelleted diet significantly exhibited an increased number of anaerobic bacteria, increased concentration of organic acids, and reduced pH in the stomach compared to fine pelleted diets [193]. Furthermore, changes in these parameters as well as a significant higher concentration of undissociated lactic acid were presumably influential in reducing *Salmonella* population in the gut of pigs.

Numerous studies have been carried out to evaluate the effects of feed structure on performance of poultry [215,218–221]. In previous reports [215,219–221], the addition of whole grains to feed instead of pelleted compound feed was also shown to increase feed conversion and growth of broilers. Furthermore, whole wheat feeding significantly increased gizzard weight, increased retention time, and reduced pH in gizzard contents compared to pellet fed birds [219,222] which in turn decreased the *Salmonella* population. In addition, uniform particle size was shown to contribute to the development and integrity of the GI tract which subsequently enhanced gut motility and backflow mechanisms in poultry including reverse peristalsis from the cloaca to the ceca [223]. Alfalfa when fed in a crumble form appears to support microflora that are accompanied by increased production of SCFA in a pattern similar to a grain-based diet [179]. While feed removal resulted in decreased fermentation capacity [30], the negative effect was neutralized by hens fed alfalfa crumbles as acetate, propionate and butyrate were observed to be the most pronounced SCFA in feces and ceca [179].

6. Conclusions

The microbial diversity of the GI ecology plays an essential role in the food animal industry and human medicine. A thorough understanding of microbial interactions can be a valid tool to prevent the environmental conditions that accompany management practices suspected in proliferating foodborne pathogens. Foodborne pathogens such as *Salmonella* possess the capability to survive in external environments during transmission from one host to the next [224]. The determination of microbial genetics and physiology associated with these mechanisms could have great potential for better control of pathogen colonization.

There have been attempts to use feed ingredients that are conducive to the growth of beneficial GI tract bacteria as well as the introduction of a bacterial population that favors optimal health and nutrition in animals to promote normal microbial growth in GI tract ecology [137]. A modulation of bacterial community in the GI tract through the use of probiotics and prebiotics remains an active research area and has shown great potential in reducing enteropathogens as well as enhancing the beneficial effects of normal microflora including *Lactobacilli* and *Bifidobacteria*. Historically, *in vitro* models, including CC-based studies, have been extensively utilized to study the ruminant GI tract ecology. More effort is needed to evaluate significant parameters of enteropathogens including steady state, nutrient status, pH status, energy requirements, and direct comparisons of metabolic and genetic responses. A better understanding of these indicators could assist in designing more novel approaches to minimize the spread of *Salmonella* in the food animal industry and decrease the consequences to human health.

References

[1] Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. *Salmonella* nomenclature. J Clin Microbiol 2000;38:2465–7.

[2] Ward LR, de Sa JD, Rowe B. A phage-typing scheme for *Salmonella* Enteritidis. Epidemiol Infect 1987;99:291–4.

[3] Varnam AH, Evans MG. *Salmonella*, foodborne pathogens, an illustrated text. Wolfe Publishing Ltd.; 1993. p. 51.

[4] Popoff MY, Bockemühl J, Gheesling LL. Supplement 2001 (No. 45) to the Kauffmann-White scheme. Res Microbiol 2003;154:173–4.

[5] Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, et al. Host adapted serotypes of *Salmonella enterica*. Epidemiol Infect 2000;125:229–55.

[6] Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607–25.

[7] Frenzen PD, Riggs TL, Buzby JC, Breuer T, Roberts T, Voetsch D, et al. *Salmonella* cost estimate update using FoodNet data. Food Rev 1999;22:10–5.

[8] Kennedy M, Villar R, Vugia DJ, Rabatsky-Ehr T, Farley MM, Pass M, et al. Hospitalizations and deaths due to *Salmonella* infections, FoodNet, 1996–1999. Clin Infect Dis 2004;38(S3):S142–8.

[9] Voetsch AC, Van Gilder TJ, Angulo FJ, Farley MM, Shallow S, Marcus R, et al. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin Infect Dis 2004;38(S3):S127–34.

[10] O'Brien SJ, de Valk H. *Salmonella*—"old" organism, continued challenges. Euro Surveill 2003;8:29–31.

[11] Anonymous. *Salmonella*: annual summary 2001. Centers for Disease Control and Prevention; 2003. p. 1–154.

[12] CDC. Preliminary foodnet data on the incidence of infection with pathogens transmitted commonly through food—10 sites, United States, 2004. MMWR 2005;54:352–6.

[13] Harrison C, Quigley C, Kaczmarski E, Devlin E. An outbreak of gastro-intestinal illness caused by eggs containing *Salmonella enteritidis* phage Type 4. J Infect 1992;24:207–10.

[14] Bryan FL, Doyle MP. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J Food Protect 1995;58:326–44.

[15] Hogue A, White P, Guard-Petter J, Schlosser W, Gast R, Ebel E, et al. Epidemiology and control of egg-associated *Salmonella* Enteritidis in the United States of America. Rev Sci Tech Int Epizoot 1997;16:542–53.

[16] Humphrey T. Chapter 15. Public health aspects of *Salmonella* infection. In: Wray C, Wray A, editors. *Salmonella* in domestic animals. Wallingford, UK: CABI Publishing; 2000. p. 245–63.

[17] Coyle EF, Palmer SR, Ribeiro CD, Jones HI, Howard AJ, Ward L, et al. *Salmonella* Enteritidis phage type 4 infection: association with hens' eggs. Lancet 1988;2:1295–7.

[18] Oldfield III EC. Emerging foodborne pathogens: keeping your patients and families safe. Rev Gastroenterol Disord 2001;1:177–86.

[19] Hennessy TW, Cheng LH, Kassenborg H, Ahuja SD, Mohle-Boetani J, Marcus R, et al. Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: a case-control study in FoodNet sites. Clin Infect Dis 2004;38(S3):S237–43.

[20] Duchet-Suchaux M, Léchopier P, Marly J, Bernardet P, Delaunay R, Pardon P. Quantification of experimental Salmonella enteritidis carrier state in B13 leghorn chicks. Avian Dis 1995;39:796–803.

[21] Suzuki S. Pathogenicity of *Salmonella enteritidis* in poultry. Int J Food Microbiol 1994;21:89–105.

[22] Lister SA. *Salmonella enteritidis* infection in broilers and broiler breeders. Vet Rec 1988;123:350.

[23] Parry SM, Palmer SR, Slader J, Humphrey T, The South East Wales Infectious Disease Liaison Group. Risk factors for salmonella food poisoning in the domestic kitchen—a case control study. Epidemiol Infect 2002;129:277–85.

[24] Humphrey TJ, Baskerville A, Mawer S, Rowe B, Hopper S. *Salmonella enteritidis* phage type 4 from the contents of intact eggs: a study involving naturally infected hens. Epidemiol Infect 1989;103:415–23.

[25] Thiagarajan D, Saeed AM, Asem EK. Mechanism of transovarian transmission of *Salmonella enteritidis* in laying hens. Poult Sci 1994;73:89–98.

[26] Holt PS. Effect of induced molting on susceptibility of White Leghorn hens to a *Salmonella enteritidis* infection. Avian Dis 1993;37:412–7.

[27] Holt PS. Horizontal transmission of *Salmonella enteritidis* in molted and unmolted laying chickens. Avian Dis 1995;39:239–49.

[28] Holt PS. Molting and *Salmonella enterica* Serovar Enteritidis infection: the problem and some solutions. Poult Sci 2003;82:1008–10.

[29] Ricke SC. The gastrointestinal tract ecology of *Salmonella* Enteritidis colonization in molting hens. Poult Sci 2003;82:1003–7.

[30] Woodward CL, Kwon YM, Kubena LF, Byrd JA, Moore RW, Nisbet DJ, et al. Reduction of *Salmonella enterica* serovar Enteritidis colonization and invasion by an alfalfa during molt in leghorn hens. Poult Sci 2005;84:185–93.

[31] Mizumoto N, Sasai K, Tani H, Baba E. Specific adhesion and invasion of *Salmonella* Enteritidis in the vagina of laying hens. Vet Microbiol 2005;111:99–105.

[32] Duchet-Suchaux M, Mompart F, Berthelot F, Beaumont C, Léchopier P, Pardon P. Differences in frequency, level, and duration of cecal carriage between four outbred chicken lines infected orally with Salmonella enteritidis. Avian Dis 1997;41:559–67.

[33] Gast RK, Holt PS. Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. Poult Sci 1998;77:1759–62.

[34] Humphrey TJ. Contamination of egg shell and contents with *Salmonella enteritidis*: a review. Int J Food Microbiol 1994;21:31–40.

[35] Barrow PA, Huggins MB, Lovell MA, Simpson JM. Observations on the pathogenesis of experimental *Salmonella typhimurium* infection in chickens. Res Vet Sci 1987;42:194–9.

[36] Turnbull PCB, Snoeyenbos GH. Experimental salmonellosis in the chicken. 1. Fate and host response in the alimentary canal, liver, and spleen. Avian Dis 1973;18:153–77.

[37] Brune K, Leffel MS, Spitznagel JK. Microbicidal activity of peroxidaseless chicken heterophile leukocytes. Infect Immun 1972;5:283–7.

[38] Stabler JG, McCormick TW, Powell KC, Kogut MH. Avian heterophils and monocytes: phagocytic and bactericidal activities against *Salmonella enteritidis*. Vet Microbiol 1994;38:293–305.

[39] Lucas RL, Lee CA. Unraveling the mysteries of virulence gene regulation in *Salmonella typhimurium*. Mol Microbiol 2000;36:1024–33.

[40] Durant JA, Corrier DE, Byrd JA, Stanker LH, Ricke SC. Feed deprivation affects crop environment and modulates *Salmonella* Enteritidis colonization and invasion of leghorn hens. Appl Environ Microbiol 1999;65:1919–23.

[41] Dunkley KD, McReynolds JL, Hume ME, Dunkley CS, Callaway TR, Kubena LF, et al. Molting in *Salmonella* Enteritidis-challenged laying hens fed alfalfa crumbles. I. *Salmonella* Enteritidis colonization and virulence gene *hilA* response. Poult Sci 2007;86:1633–9.

[42] Barnes EM, Mead GC, Barnum DA, Harry EG. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br Poult Sci 1972;13:311–26.

[43] Rozee KR, Cooper D, Lam K, Costerman JW. Microbial flora of the mouse ileum mucus layer and epithelial surface. Appl Environ Microbiol 1982;43:1451–63.

[44] Chase DG, Erlandsen SL. Evidence for a complex life cycle and endospore formation in attached, filamentous segmented bacterium from murine ileum. J Bacteriol 1976;127:572–83.

[45] Phillips M, Lee A, Leach WD. The mucosa-associated microflora of the rat intestine: a study of normal distribution and magnesium sulphate induced diarrhoea. Aust J Exp Biol Med Sci 1978;56:649–62.

[46] Suegara N, Morotomi M, Watanabe T, Kawai Y, Matai M. Behavior of microflora in the rat stomach: adhesion of lactobacilli to the keratinized epithelial cells of the rat stomach in vitro. Infect Immun 1975;12:173–9.

[47] Ricke SC, Pillai SD. Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. Crit Rev Microbiol 1999;25:19–38.

[48] Józefiak D, Rutkowski A, Martin SA. Carbohydrates fermentation in the avian ceca. Anim Feed Sci Technol 2004;113:1–15.

[49] Ricke SC, Woodward CL, Kwon YM, Kubena LF, Nisbet DJ. Limiting avian gastrointestinal tract *Salmonella* colonization by cecal anaerobic bacteria, and a potential role for methanogens. In: Beier RC, Pillai SD, Phillips TD, editors. Preharvest and postharvest food safety: contemporary issues and future directions. Ames, IA: Blackwell Publishing Professional; 2004. p. 141–50.

[50] Saengkerdsub S, Kim WK, Anderson RC, Nisbet DJ, Ricke SC. Effects of nitrocompounds and feed stuffs on in vitro methane production in chicken cecal contents and rumen fluid. Anaerobe 2006;12:85–92.

[51] Saengkerdsub S, Herrera P, Woodward CL, Anderson RC, Nisbet DJ, Ricke SC. Detection of methane and quantification of methanogenic archaea in faeces from young broiler chickens using real-time PCR. Lett Appl Microbiol 2007;45:629–34.

[52] Saengkerdsub S, Anderson RC, Wilkinson HH, Kim W, Nisbet DJ, Ricke SC. Identification and quantification of methanogenic archaea in adult chicken ceca. Appl Environ Microbiol 2007;73:353–6.

[53] Annison EF, Hill KJ, Kenworthy R. Volatile fatty acids in the digestive tract of the fowl. Br J Nutr 1968;22:207–16.

[54] Barnes EM. The intestinal microflora of poultry and game birds during life and after storage. J Appl Bacteriol 1979;46:407–19.

[55] Barnes EM, Mead GC, Griffiths NM. The microbiology and sensory evaluation of pheasants hung at 5, 10 and 15 °C. Br Poult Sci 1973;14:229–40.

[56] Barnes EM, Impey CS, Stevens BJH. Factors affecting the incidence and antisalmonella activity of the anaerobic cecal flora of the young chicken. J Hyg 1979;82:263–83.

[57] Zhu XY, Zhong T, Pandya Y, Joerger RD. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl Environ Microbiol 2002;68:124–37.

[58] Isaacson HR, Hinds FC, Bryant MP, Owens FN. Efficiency of energy utilization by mixed rumen bacteria in the continuous culture. J Dairy Sci 1975;58:1645–59.

[59] Russell JB. Heat production by ruminal bacteria in continuous culture and its relationship to maintenance energy. J Bacteriol 1986;168:694–701.

[60] Russell JB, Cook GM. Energetics of bacterial growth: balance of anabolic and catabolic reactions. Microbiol Rev 1995;59:48–62.

[61] Ushijima T, Seto A. Selected faecal bacteria and nutrients essential for antagonism of *Salmonella typhimurium* in anaerobic continuous flow cultures. J Med Microbiol 1991;35:111–7.

[62] Coleman ME, Dressen DW, Wiegert RG. A simulation of microbial competition in the human colonic ecosystem. Appl Environ Microbiol 1996;62:3632–9.

[63] Hume ME, Nisbet DJ, DeLoach JR. In vitro ^{14}C-amino acid fermentation by CF3™, a characterized continuous-flow competitive exclusion culture of caecal bacteria. J Appl Microbiol 1997;83:236–42.

[64] Nisbet DJ, Anderson RC, Corrier DE, Harvey RB, Stanker LH. Modeling the survivability of *Salmonella typhimurium* in the chicken cecae using an anaerobic continuous-culture of chicken cecal bacteria. Microb Ecol Health Dis 2000;12:42–7.

[65] Hoover WH, Crooker BA, Sniffen CJ. Effects of differential solid–liquid removal rates on protozoa numbers in continuous cultures of rumen contents. J Anim Sci 1976;43:528–34.

[66] Schlecht E, Richter H, Fernández-Rivera S, Becker K. Gastrointestinal passage of Sahelian roughages in cattle, sheep and goats, and implications for livestock-mediated nutrient transfers. Anim Feed Sci Technol 2007;137:93–114.

[67] Van Weyenberg S, Sales J, Janssens GPJ. Passage rate of digesta through the equine gastrointestinal tract: a review. Livestock Sci 2006;99:3–12.

[68] Duke GE. Avian digestion. In: Duke GE, editor. Dukes' physiology of domestic animals. Ithaca and London: Cornell University Press; 1977. p. 313–20.

[69] Amerah AM, Ravindran V, Lentle RG, Thomas DG. Feed particle size: implications on the digestion and performance of poultry. World's Poult Sci J 2007;63:439–55.

[70] Nisbet D. Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. Antonie van Leeuwenhoek 2002;81:481–6.

[71] Ha SD, Ricke SC, Nisbet DJ, Corrier DE, DeLoach JR. Serine utilization as a potential competition mechanism between *Salmonella* and a chicken cecal bacterium. J Food Protect 1994;57:1074–9.

[72] Ha SD, Nisbet DJ, Corrier DE, DeLoach JR, Ricke SC. Comparison of *Salmonella typhimurium* and selected facultative chicken cecal bacteria survivability after specific amino acid-limited batch growth. J Food Prot 1995;58:1335–9.

[73] Dunkley KD, Callaway TR, Chalova VI, Anderson RC, Kundinger MM, Dunkley CS, et al. Growth and genetic responses of *Salmonella* Typhimurium to pH-shifts in an anaerobic continuous culture. Anaerobe 2008;14:35–42.

[74] Kolter R. The stationary phase of the bacterial life cycle. Annu Rev Microbiol 1993;47:855–74.

[75] Siegele DH, Kolter R. Life after log. J Bacteriol 1992;174:345–8.

[76] Almirón M, Link AJ, Furlong D, Kolter R. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev 1992;6:2646–54.

[77] Foster JW, Spector MP. Phosphate starvation regulon of *Salmonella typhimurium*. J Bacteriol 1986;166:666–9.

[78] Gentry DR, Hernandez VJ, Nguyen LH, Jensen DB, Cashel M. Synthesis of the stationary-phase sigma factor σ^s is positively regulated by ppGpp. J Bacteriol 1993;175:7982–9.

[79] Koch AL. The adaptive responses of *Escherichia coli* to a feast and famine existence. Adv Microb Physiol 1971;6:147–217.

[80] Lange R, Hengge-Aronis R. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol Microbiol 1991;5:49–59.

[81] O'Neal CR, Gabriel WM, Turk AK, Libby SJ, Fang FC, Spector MP. RpoS is necessary for both positive and negative regulation of starvation survival genes during phosphate, carbon, and nitrogen starvation in *Salmonella typhimurium*. J Bacteriol 1994;176:4610–6.

[82] Ševčík M, Šebková A, Volf J, Rychlík I. Transcription of *arcA* and *rpoS* during growth of *Salmonella typhimurium* under aerobic and microaerobic conditions. Microbiology 2001;147:701–8.

[83] Giannella RA, Washington O, Gemski P, Formal SB. Invasion of HeLa cells by *Salmonella typhimurium*: a model for study of invasiveness of *Salmonella*. J Infect Dis 1973;128:69–79.

[84] Finlay BB, Gumbiner B, Falkow S. Penetration of Salmonella through a polarized Madin-Darby canine kidney epithelial monolayer. J Cell Biol 1988;107:221–30.

[85] Betts J, Finlay BB. Identification of Salmonella typhimurium invasiveness loci. Can J Microbiol 1992;38:852–7.

[86] Galán JE, Curtiss III R. Cloning and molecular characterization of genes whose products allow Salmonella typhimurium to penetrate tissue culture cells. Proc Natl Acad Sci USA 1989;86:6383–7.

[87] Collazo CM, Galán JE. The invasion-associated type-III protein secretion system in Salmonella—a review. Gene 1997;192:51–9.

[88] Ochman H, Groisman EA. Distribution of pathogenicity islands in Salmonella spp. Infect Immun 1996;64:5410–2.

[89] Rodriquez CR, Schechter LM, Lee CA. Detection and characterization of the Salmonella typhimurium HilA protein. BMC Microbiol 2002;2:31.

[90] Durant JA, Lowry VK, Nisbet DJ, Stanker LH, Corrier DE, Ricke SC. Short-chain fatty acids affect cell-association and invasion of HEp-2 cells by Salmonella typhimurium. J Environ Sci Health 1999;B34:1083–99.

[91] Durant JA, Lowry VK, Nisbet DJ, Stanker LH, Corrier DE, Ricke SC. Short chain fatty acids alter HEp-2 cell association and invasion by stationary growth phase Salmonella typhimurium. J Food Sci 2000;65:1206–9.

[92] Durant JA, Lowry VK, Nisbet DJ, Stanker LH, Corrier DE, Ricke SC. Late logarithmic Salmonella typhimurium HEp-2 cell association and invasion response to short-chain fatty acid addition. J Food Safety 2000;20:1–11.

[93] Hengge-Aronis R. Survival of hunger and stress: the role of rpoS in early stationary phase gene expression in E. coli. Cell 1993;72:165–8.

[94] Lange R, Barth M, Hengge-Aronis R. Complex transcriptional control of the σ^S-dependent stationary-phase-induced and osmotically regulated osmY (csi-5) gene suggests novel roles for Lrp, cyclic AMP (cAMP) receptor protein-cAMP complex, and integration host factor in the stationary-phase response of Escherichia coli. J Bacteriol 1993;175:7910–7.

[95] Kowarz L, Coynault C, Robbe-Saule V, Norel F. The Salmonella typhimurium katf (rpoS) gene: cloning, nucleotide sequence, and regulation of spvR and spvABCD virulence plasmid genes. J Bacteriol 1994;176:6852–60.

[96] Robbe-Saule V, Coynault C, Norel F. The live oral vaccine Ty21a is a rpoS mutant and is susceptible to various environmental stresses. FEMS Microbiol Lett 1995;126:171–6.

[97] Hengge-Aronis R. Interplay of global regulators and cell physiology in the general stress response of Escherichia coli. Curr Opin Microbiol 1999;2:148–52.

[98] Ishihama A. Adaptation of gene expression in stationary phase bacteria. Curr Opin Genet Dev 1997;7:582–8.

[99] Loewen PC, Hu B, Strutinsky J, Sparling R. Regulation of the rpoS regulon of Escherichia coli. Can J Microbiol 1998;44:707–17.

[100] Jishage M, Kvint K, Shingler V, Nyström T. Regulation of σ factor competition by the alarmone ppGpp. Gene Dev 2002;16:1260–70.

[101] Komitopoulou E, Baintob NJ, Adams MR. Oxidation–reduction potential regulates RpoS levels in Salmonella Typhimurium. Appl Microbiol 2004;96:271–8.

[102] Hirsch M, Elliot T. Fis regulates transcriptional induction of RpoS in Salmonella enterica. J Bacteriol 2005;187:1568–80.

[103] Robey M, Benito A, Hutson RH, Pascaul C, Park SF, Mackey BM. Variation in resistance to high hydrostatic pressure and rpoS heterogenicity in natural isolates of Escherichia coli O157:H7. Appl Environ Microbiol 2001;67:4901–7.

[104] Fierer J, Kruse M, Tauxe M, Guineu DG. Salmonella typhimurium bacteremia: association with the virulence plasmid. J Infect Dis 1992;166:639–42.

[105] Ibanez-Ruiz M, Robbe-Saule V, Hermant D, Labrude S, Norel F. Identification of rpoS (σ^s)-regulated genes in Salmonella enterica serovar Typhimurium. J Bacteriol 2000;182:5749–56.

[106] Coynault C, Robbe-Saule V, Popoff MY, Norel F. Growth phase and SpvR regulation of transcription of Salmonella typhimurium spvABC virulence genes. Microb Pathog 1992;13:133–43.

[107] Foster JW. Low pH adaptation and the acid tolerance response of Salmonella typhimurium. Crit Rev Microbiol 1995;21:215–37.

[108] Foster JW, Hall HK. Inducible pH homeostasis and the acid tolerance response of Salmonella typhimurium. J Bacteriol 1991;173:5129–35.

[109] Hall HK, Karem K, Foster JW. Molecular responses of microbes to environmental pH stress. Adv Microb Physiol 1995;37:229–72.

[110] Lee IS, Lin J, Hall HK, Bearson B, Foster JW. The stationary stage sigma factor σ^s (Rpos) is required for a sustained acid tolerance in virulent Salmonella typhimurium. Mol Microbiol 1995;17:155–67.

[111] Lee IS, Slonczewski JL, Foster JW. A low-pH-inducible-stationary-phase acid tolerance response in Salmonella typhimurium. J Bacteriol 1994;176:1422–6.

[112] Foster JW. Salmonella acid shocked proteins are required for the adaptive acid tolerance response. J Bacteriol 1991;173:6896–902.

[113] Bearson BL, Wilson L, Foster JW. A low pH-inducible, PhosPQ-dependent acid tolerance response protects Salmonella typhimurium against inorganic acid stress. J Bacteriol 1998;180:2409–17.

[114] Foster JW. The acid tolerance response of Salmonella typhimurium involves transient synthesis of key acid shock proteins. J Bacteriol 1993;175:1981–7.

[115] Hungate RE. The rumen and its microbes. New York, NY: Academic Press; 1966.

[116] Kass ML, Van Soest PJ, Pond WG. Utilization of dietary fiber from alfalfa by growing swine. II. Volatile fatty acid concentrations in and disappearance from the gastrointestinal tract. J Anim Sci 1980;50:192–7.

[117] Jamroz D, Wiliczkiewicz A, Skorupiñska J. Fermentation and apparent digestion of the structural carbohydrates in chicks, ducks and geese fed triticale mixtures supplemented with enzymes. J Anim Physiol Anim Nutr 1998;79:1–17.

[118] Gasaway WC. Seasonal variation in diet, volatile fatty acid production and size of the cecum of rock ptarmigan. Comp Biochem Physiol 1976;A53:104–9.

[119] Gasaway WC. Volatile fatty acids and metabolizable energy derived from cecal fermentation in the willow ptarmigan. Comp Biochem Physiol 1976;A53:115–21.

[120] Van der Wielen PWJJ, Biesterveld S, Notermans S, Hofstra H, Urlings BAP, Van Knapen F. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl Environ Microbiol 2000;66:2536–40.

[121] Corrier DE, Hinton Jr A, Ziprin RL, Beier RC, DeLoach JR. Effect of dietary lactose on cecal pH, bacteriostatic volatile fatty acids, and Salmonella typhimurium colonization of broiler chicks. Avian Dis 1990;34:617–25.

[122] Nurmi E, Rantala M. New aspects of Salmonella infection in broiler production. Nature 1973;241:210–1.

[123] Van der Wielen PWJJ, Biesterveld S, Lipman LJA, Van Knapen F. Inhibition of a glucose-limited sequencing fed-batch culture of Salmonella enterica serovar Enteritidis by volatile fatty acids representative of the ceca of broiler chickens. Appl Environ Microbiol 2001;67:1979–82.

[124] Kwon YM, Ricke SC. Induction of acid resistance of Salmonella typhimurium by exposure to short-chain fatty acids. Appl Environ Microbiol 1998;64:3458–63.

[125] Kwon YM, Woodward CL, Pillai SD, Peña J, Corrier DE, Byrd JA, et al. Litter and aerosol sampling of chicken houses for rapid detection of Salmonella typhimurium contamination using gene amplification. J Ind Microbiol Biotechnol 2000;24:379–82.

[126] Durant JA, Corrier DE, Ricke SC. Short-chain volatile fatty acids modulate the expression of the hilA and invF genes of Salmonella Typhimurium. J Food Prot 2000;63:573–8.

[127] Ricke SC. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult Sci 2003;82:632–9.

[128] Van der Wielen PWJJ, Lipman LJA, van Knapen F, Biesterveld S. Competitive exclusion of Salmonella enterica serovar Enteritidis by Lactobacillus crispatus and Clostridium lactatifermentans in a sequencing fed-batch culture. Appl Environ Microbiol 2002;68:555–9.

[129] Cherrington CA, Hinton M, Pearson GR, Chopra I. Inhibition of Escherichia coli K12 by short-chain organic acid: lack of evidence of induction of the SOS response. J Appl Bacteriol 1991;70:156–60.

[130] Russell JB. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J Appl Bacteriol 1992;73:363–70.

[131] McHan F, Shotts EB. Effects of short-chain fatty acids on the growth of Salmonella typhimurium in an in vitro system. Avian Dis 1993;37:396–8.

[132] Gedek B. Probiotika in der Tierernahrung Wirkungen auf Leistung und Tiergesunheit. Kraftfutter: Zeitschrift fuer die Futtermittel und Getreidwirtschaft 1986;3:80–4.

[133] Fuller R. Probiotics in man and animals. A review. J Appl Bacteriol 1989;66:365–78.

[134] Dibner JJ, Richards JD, Knight CD. Microbial imprinting in gut development and health. J Appl Poult Res 2008;17:174–88.

[135] Berge RD, Savage DC. Immune responses of specific pathogen-free and gnotobiotic mice to antigens of indigenous and nonindigenous microorganisms. Infect Immun 1975;11:320–9.

[136] Tlaskalova-Hogenova H, Mandel L, Trebichavsky I, Kovaru F, Barot R, Sterzl J. Development of immune responses in early pig ontogeny. Vet Immunol Immunopathol 1994;43:135–42.

[137] Jin LZ, Ho YW, Abdullah N, Jalaludin S. Probiotics in poultry: modes of action. Worlds Poult Sci J 1997;53:351–68.

[138] Tellez G, Higgins SE, Donoghue AM, Hargis BM. Digestive physiology and the role of microorganisms. J Appl Poult Res 2006;15:136–44.

[139] Nisbet DJ, Corrier DE, DeLoach JR. Effect of mixed cecal microflora maintained in continuous culture and of dietary lactose on Salmonella typhimurium colonization in broiler chicks. Avian Dis 1993;37:528–35.

[140] Nisbet DJ, Ricke SC, Scanlan CM, Corrier DE, Hollister AG, DeLoach JR. Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilities early cecal bacterial colonization and increases resistance to Salmonella. J Food Protect 1994;57:12–5.

[141] Steer T, Carpenter H, Tuohy K, Gibson GR. Perspectives on the role of the human gut microbiota and its modulation by pro- and pre-biotics. Nutr Res Rev 2000;13:229–54.

[142] Revolledo L, Ferreira AJP, Mead GC. Prospects in Salmonella control: competitive exclusion, probiotics, and enhancement of avian intestinal immunity. J Appl Poult Res 2006;15:341–51.

[143] Stavric S, D'Aoust J-Y. Undefined and defined bacterial preparations for competitive exclusion of Salmonella in poultry. J Food Prot 1993;56:173–80.

[144] Doyle MP, Erickson MC. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult Sci 2006;85:960–73.

[145] Nisbet DJ, Corrier DE, Ricke SC, Hume ME, Byrd II JA, DeLoach JL. Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to Salmonella in chicks. Anaerobe 1996;2:345–50.

[146] Hinton Jr A, Corrier DE, Spates GE, Norman JO, Ziprin RL, Beier RC, et al. Biological control of Salmonella typhimurium in young chickens. Avian Dis 1990;34:626–33.

[147] Corrier DE, Hargis B, Hinton Jr A, Lindsey D, Caldwell D, Manning J, et al. Effect of anaerobic cecal microflora and dietary lactose on colonization resistance of layer chicks to invasive Salmonella enteritidis. Avian Dis 1991;35:337–43.

[148] Durant JA, Nisbet DJ, Ricke SC. Response of selected poultry cecal probiotic bacteria and a primary poultry Salmonella typhimurium isolate grown with or without glucose in liquid batch culture. J Environ Sci Health 2000;B35: 503–16.

[149] Corrier DE, Nisbet DJ. Competitive exclusion in the control of Salmonella enterica serovar Enteritidis infection in laying poultry. Chapter 35. In: Saeed AM, Gast RK, Potter ME, Wall PG, editors. Salmonella enterica serovar Enteritidis in human and animals: epidemiology, pathogenesis, and control. Ames, Iowa: Iowa State University Press; 1999. p. 391–6.

[150] Watkins BA, Miller BF. Competitive gut exclusion of avian pathogens by Lactobacillus acidophilus in gnotobiotic chicks. Poultry Sci 1983;62: 1772–979.

[151] Stavric S, Gleeson TM, Blanchfield B. Effect of avian intestinal microflora possessing adhering and hydrophobic properties on competitive exclusion of Salmonella typhimurium from chicks. J Appl Bacteriol 1991;70:414–21.

[152] Soerjadi AS, Rufner R, Snoeyenbos GH, Weinack OM. Adherence of salmonellae and native gut microflora to the gastrointestinal mucosa of chicks. Avian Dis 1982;26:576–84.

[153] Droleskey RE, Corrier DE, Nisbet DJ, DeLoach JR. Colonization of cecal mucosal epithelium in chicks treated with a continuous flow culture of 29 characterized bacteria: confirmation by scanning electron microscopy. J Food Prot 1995;58:837–42.

[154] Macfarlane GT, Steed H, Macfarlane S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J Appl Microbiol 2008;104:305–44.

[155] Patterson JA, Burkholder KM. Application of prebiotics and probiotics in poultry production. Poultry Sci 2003;82:627–31.

[156] Roller M, Rechkemmer G, Watzl B. Prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis modulates intestinal immune functions in rats. J Nutr 2004;134:153–6.

[157] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125:1401–12.

[158] Cummings JH, Macfarlane GT, Englyst HN. Prebiotic digestion and fermentation. Am J Clin Nutr 2001;73:415S–20S.

[159] Bach Knudsen KE. The nutritional significance of "dietary fibre" analysis. Anim Feed Sci Technology 2001;90:3–20.

[160] Wenk C. The role of dietary fibre in the digestive physiology of the pig. Anim Feed Sci Technol 2001;90:21–33.

[161] Griggs JP, Jacob JP. Alternatives to antibiotics for organic poultry production. J Appl Poult Res 2005;14:750–6.

[162] Cummings JH, McFarlane GT. Gastrointestinal effects of prebiotics. Br J Nutr 2002;87(Suppl. 2):S145–51.

[163] Bedford M. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. World Poultry Sci J 2000;56:347–65.

[164] Mitsuoka T, Hidaka H, Eida T. Effects of fructooligosaccharides on intestinal microflora. Die Nahrung 1987;31:427–36.

[165] Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Sci 2003;82:1030–6.

[166] Bailey JS, Blankenship LC, Cox NA. Effect of fructooligosaccharide on Salmonella colonization of the chicken intestine. Poultry Sci 1991;70:2433–8.

[167] Bomba A, Nemcová R, Gancarčíková S, Herich R, Guba P, Mudroňová D. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrin, fructo-oligosaccharides and polyunsaturated fatty acids. Brit J Nutr 2002;88(Suppl.):S95–9.

[168] Donalson LM, Kim WK, Chalova VI, Herrera P, McReynolds JL, Gotcheva VG, et al. In vitro fermentation response of laying hen cecal bacteria to combinations of fructooligosaccharide (FOS) prebiotic with alfalfa or layer ration. Poult Sci 2008;87:1263–75.

[169] Donalson LM, Kim WK, Chalova VI, Herrera P, Woodward CL, McReynolds JL, et al. In vitro anaerobic incubation of Salmonella enterica serotype Typhimurium and laying hen cecal bacteria in poultry feed substrates and a fructooligosaccharide prebiotic. Anaerobe 2007;13:208–14.

[170] Donalson LM, McReynolds JL, Kim WK, Chalova VI, Woodward CL, Kubena LF, et al. The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract fermentation, Salmonella Enteritidis infection and intestinal shedding in laying hens. Poult Sci 2008;87:1253–62.

[171] Kuenzel WJ. Neurobiology of molt in avian species. Poult Sci 2003;82:981–91.

[172] Bell DD. Historical and current molting practices in the US table egg industry. Poult Sci 2003;82:965–70.

[173] Brake J. Recent advances in induced molting. Poult Sci 1993;72:929–31.

[174] Holt PS, Macri NP, Porter Jr RE. Microbiological analysis of early Salmonella enteritidis infection in molted and unmolted hens. Avian Dis 1995;39:55–63.

[175] Webster AB. Physiology and behavior of the hen during induced molt. Poult Sci 2003;82:992–1002.

[176] Park SY, Kim WK, Birkhold SG, Kubena LF, Nisbet DJ, Ricke SC. Induced moulting issues and alternative dietary strategies for the egg industry in the United States. World's Poult Sci J 2004;60:196–209.

[177] Anderson KE, Havenstein GB. Effects of alternative molting programs and population on layer performance: results of the thirty-fifth North Carolina layer performance and management test. J Appl Poult Res 2007;16:365–80.

[178] Petek M, Gezen SS, Alpay F, Cibik R. Effects of non-feed removal molting methods on egg quality traits in commercial brown egg laying hens in Turkey. Trop Anim Health Prod 2007;. doi:10.1007/s11250-007-9102-5.

[179] Dunkley KD, McReynolds JL, Hume ME, Dunkley CS, Callaway TR, Kubena LF, et al. Molting in Salmonella Enteritidis-challenged laying hens fed alfalfa crumbles. II. Fermentation and microbial ecology response. Poult Sci 2007;86:2101–9.

[180] Kaplan H, Hutkins RW. Fermentation of fructooligosaccharides by lactic acid bacteria and Bifidobacteria. Appl Environ Microbiol 2000;66:2682–4.

[181] Fernandez F, Hinton M, Van Gils B. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella Enteritidis colonization. Avian Pathol 2002;31:49–58.

[182] van den Broek LAM, Hinz SWA, Beldman G, Vincken J-P, Voragen AGJ. Bifidobacterium carbohydrases—their role in breakdown and synthesis of (potential) prebiotics. Mol Nutr Food Res 2007;52:146–63.

[183] Holt PS. Effects of induced moulting on immune responses of hens. Br Poult Sci 1992;33:165–75.

[184] Holt PS. Effect of induced molting on B cell and CT4 and CT8T cell numbers in spleens and peripheral blood of white leghorn hens. Poult Sci 1992;71: 2027–34.

[185] Dunkley CS, McReynolds JL, Dunkley KD, Njongmeta LN, Berghman LR, Kubena LF, et al. Molting in Salmonella Enteritidis-challenged laying hens fed alfalfa crumbles. IV. Immune and stress protein response. Poult Sci 2007;86:2502–8.

[186] Dunkley CS, McReynolds JL, Dunkley KD, Kubena LF, Nisbet DJ, Ricke SC. Molting in Salmonella Enteritidis-challenged laying hens fed alfalfa crumbles. III. Blood plasma metabolite response. Poult Sci 2007;86:2492–501.

[187] Kim WK, Donalson LM, Herrera P, Kubena LF, Nisbet DJ, Ricke SC. Comparisons of molting diets on skeletal quality and eggshell parameters in hens at the end of the second egg-laying cycle. Poult Sci 2005;84:522–7.

[188] Kim WK, Donalson LM, Mitchell AD, Kubena LF, Nisbet DJ, Ricke SC. Effects of alfalfa and fructooligosaccharide on molting parameters and bone qualities using dual energy X-ray absorptiometry and conventional bone assays. Poult Sci 2006;85:15–20.

[189] Kim WK, Donalson LM, Bloomfield SA, Hogan HA, Kubena LF, Nisbet DJ, et al. Molt performance and bone density of cortical, medullary, and cancellous bone in laying hens during feed restriction or alfalfa-based feed molt. Poult Sci 2007;86:1821–30.

[190] Holt PS, Porter Jr RE. Microbiological and histopathological effects of an induced-molt fasting procedure on a Salmonella enteritidis infection in chickens. Avian Dis 1992;36:610–8.

[191] Brant CS, Thacker EJ. A concept of rate of food passage through the gastrointestinal tract. J Anim Sci 1958;17:218–23.

[192] Dänicke S, Simon O, Jeroch H, Bedford M. Interaction between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 1. Physio-chemical chyme features. Br Poult Sci 1997;38:537–45.

[193] Mikkelsen LL, Naughton PJ, Hedemann MS, Jensen BB. Effects of physical properties of feed on microbial ecology and survival of Salmonella enterica serovar Typhimurium in the pig gastrointestinal tract. Appl Environ Microbiol 2004;70:3485–92.

[194] McNab J. The avian caeca: a review. World's Poult Sci J 1973;29:251–63.

[195] Keshavarz K, Quimby FW. An investigation of different molting techniques with an emphasis on animal welfare. J Appl Poult Res 2002;11:54–67.

[196] Davis AJ, Lordelo MM, Dale N. The use of cottonseed meal with or without added soapstock in laying hen diets. J Appl Poult Res 2002;11:127–33.

[197] Seo K-H, Holt PS, Gast RK. Comparison of Salmonella Enteritidis infection in hens molted via long-term feed withdrawal versus full-fed wheat middling. J Food Prot 2001;64:1917–21.

[198] Landers KL, Howard ZR, Woodward CL, Birkhold SG, Ricke SC. Potential of alfalfa as an alternative molt induction diet for laying hens: egg quality and consumer acceptability. Bioresour Technol 2005;96:907–11.

[199] Landers KL, Moore RW, Dunkley CS, Herrera P, Kim WK, Landers DA, et al. Immunological cell and serum metabolite response of 60-week-old commercial laying hens to an alfalfa meal molt diet. Bioresour Technol 2008;99:604–8.

[200] Landers KL, Moore RW, Herrera P, Landers DA, Howard ZR, McReynolds JL, et al. Organ weight and serum triglyceride responses of older (80 week) commercial laying hens fed an alfalfa meal molt diet. Bioresour Technol 2008;99:6692–6.

[201] Landers KL, Woodward CL, Li X, Kubena LF, Nisbet DJ, Ricke SC. Alfalfa as a single dietary source for molt induction in laying hens. Bioresour Technol 2005;96:565–70.

[202] McReynolds J, Kubena L, Byrd J, Anderson R, Ricke S, Nisbet D. Evaluation of Salmonella enteritidis in molting hens after administration of an experimental chlorate product (for none days) in the drinking water and feeding an alfalfa molt diet. Poult Sci 2005;84:1186–90.

[203] McReynolds JL, Moore RW, Kubena LF, Byrd JA, Woodward CL, Nisbet DJ, et al. Effect of various combinations of alfalfa and standard layer diet on susceptibility of laying hens to Salmonella Enteritidis during forced molt. Poult Sci 2006;85:1123–8.

[204] Tsukahara T, Ushida K. Effects of animal or plant proteins diets on cecal fermentation in guinea pigs (Cavia porcellus), rats (Rattus norvegicus) and

chicks (*Gallus gallus domesticus*). Comp Biochem Physiol A Mol Integr Physiol 2000;127:139–46.

[205] Allen VM, Fernandez F, Hinton MH. Evaluation of the influence of supplementing the diet with mannose or palm kernel meal on salmonella colonisation in poultry. Br Poult Sci 1997;38:485–8.

[206] Dunkley KD, Dunkley CS, Njongmeta NL, Callaway TR, Hume ME, Kubena LF, et al. Comparison of in vitro fermentation and molecular microbial profiles of high-fiber feed substrates incubated with chicken cecal inocula. Poult Sci 2007;86:801–10.

[207] Falcão-e-Cunha L, Peres HB, Freire JPB, Castro-Solla L. Effects of alfalfa, wheat bran or beet pulp, with or without sunflower oil, on caecal fermentation and on digestibility in the rabbit. Anim Feed Sci Technol 2004;117:131–49.

[208] Ponte PIP, Mendes I, Quaresma M, Aguiar MNM, Lemos JPC, Ferreira LMA, et al. Cholesterol levels and sensory characteristics of meat from broilers consuming moderate to high levels of alfalfa. Poult Sci 2004;83:810–4.

[209] Ponte PIP, Ferreira LMA, Soares MAC, Aguiar MANM, Lemos JPC, Mendes I, et al. Use of cellulases and xylanases to supplement diets containing alfalfa for broiler chicks: effects on bird performance and skin color. J Appl Poult Res 2004;13:412–20.

[210] Oleszek W. Alfalfa saponins: structure, biological activity, and chemotaxonomy. In: Waller GR, Yamasaki K, editors. Saponins used in food and agriculture. New York: Plenum Press; 1996. p. 155–70.

[211] Sibbald IR. Passage of feed through the adult rooster. Poult Sci 1979;58:446–59.

[212] Sen S, Makkar HPS, Becker K. Alfalfa saponins and their implications in animal nutrition. J Agric Food Chem 1998;46:131–40.

[213] Barnes EM, Impey CS, Cooper DM. Manipulation of the crop and intestinal flora of the newly hatched chick. Am J Clin Nutr 1980;33:2426–33.

[214] Apajalahti J, Kettunen A, Graham H. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult Sci J 2004;60:223–32.

[215] Svihus B, Herstad O, Newman CW, Newman RK. Comparison of performance and intestinal characteristics of broiler chickens fed on diets containing whole, rolled or ground barley. Br Poult Sci 1997;38:524–9.

[216] Nir L, Hillel R, Shefet G, Nitsan Z. Effects of grain particle size on performance. 2. Grain texture interactions. Poult Sci 1994;73:781–91.

[217] Nir I, Twina Y, Grossman E, Nitsan Z. Quantitative effects of pelleting on performance, gastrointestinal tract and behaviour of meat-type chickens. Br Poult Sci 1994;35:589–602.

[218] Engberg RM, Hedemann MS, Jensen BB. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br Poult Sci 2002;43:569–79.

[219] Engberg RM, Hedemann MS, Steenfeldt S, Jensen BB. The influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult Sci 2004;83:925–38.

[220] Jones GPD, Taylor RD. The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. Br Poult Sci 2001;42:477–83.

[221] Svihus B, Hetland H. Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Br Poult Sci 2001;42:633–7.

[222] Bjerrum J, Pedersen K, Engberg RM. The influence of whole wheat feeding on Salmonella infection and gut flora composition in broilers. Avian Dis 2005;49:9–15.

[223] Karasawa Y. Effects of colostomy on nitrogen nutrition in the chicken fed a low protein diet plus urea. J Nutr 1989;119:1388–91.

[224] Winfield MD, Groisman EA. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl Environ Microbiol 2003;69:3687–94.